IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Waldmann *et al.* Atty Dkt No.: 1324.028

Serial No.: Unknown, Continuation of PCT/GB99/03653
International Filing Date: 05 November 1999
Priority Data: GB 9824306.6 05 November 1998

Title: METHOD FOR PRODUCING DENDRITIC CELLS

Assistant Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

PRELIMINARY AMENDMENT UNDER 37 CFR 1.115

Dear Sir:

This paper is filed contemporaneously with a filing under 37 CFR 1.53(b). Prior to examination of this continuation application, please amend the priority application as follows:

A. In the specification:

1.) Page 1, please insert the following paragraph after the title:

-- Cross Reference to Related Applications

This application is a continuation of co-pending International Patent Application Number PCT/GB99/03653, filed November 5, 1999, and claims priority from GB Patent Application Number 9824306.6, filed November 5, 1998. The entire disclosures of the prior applications are incorporated herein by reference.

Field of the Invention --

2.) Page 6, please replace paragraph 6 (lines 20-24) with:

-- In another aspect, the invention provides a method for investigating a mammalian gene, which method comprises generating a test population of dendritic cells from a population of embryonic stem cells and comparing the test dendritic cells in respect of the gene.

Brief Description of the Drawings --

3.) Page 7, please replace paragraph 1 (lines 1-2) with:

-- Figure 10 shows the generation of esDC stably transfected with GFP following introduction of the transgene into the parent ES cell line.

Detailed Description of the Invention --

4.) Page 25, please replace paragraph 1 (line 1) with:

-- Claims

We claim: --

5.) Please insert the annexed new page 29, containing the following:

-- Abstract of the Invention

Disclosed are embryonic stem cell-derived dendritic cells, genetically modified immature dendritic cells capable of maturation, as well as methods for the production of such cells. In one embodiment, the cells made be produced by a method comprising the steps of providing a population of embryonic stem cells; culturing the embryonic stem cells in the presence of a cytokine or combination of cytokines which brings about differentiation of the embryonic stem cells into dendritic cells; and recovering the dendritic cells from the culture. In a further embodiment, the cells may be genetically modified. --

B. In the claims:

1.) Canceled claims

Please cancel claims 1-63, without prejudice.

2.) New claims

Please add new claims 64-104 as indicated below.

Clean Version of Pending Claims

64. A process for producing a long-term culture of immature dendritic cells, which process comprises:

(i) providing a population of embryonic stem cells;

(ii) culturing the embryonic stem cells in the presence of a cytokine or combination of cytokines which bring about differentiation of the embryonic stem cells into immature dendritic cells to produce a long-term culture of immature dendritic cells; and

(iii) recovering immature dendritic cells from the culture, which immature dendritic cells are capable of maturation to an immunostimulatory phenotype.

65. The process of claim 64 further comprising the step (iv) of stimulating the immature dendritic cells to mature thereby producing mature immunostimulatory dendritic cells.

66. The process of claim 65 wherein the immature dendritic cells are stimulated to mature with an inflammatory mediator.

67. The process of claim 65 wherein the inflammatory mediator is LPS.

68. The process according to claim 64, wherein the cytokine or combination of cytokines is or includes IL-3.

69. he process according to claim 68, wherein a combination of cytokines including IL-3 and GM-CSF is used.

70. The process according to claim 64, wherein the embryonic stem cells in (i) are in the form of embryoid bodies.

71. The process according to claim 64, wherein the embryonic stem cells are genetically modified.

72. The process of claim 71, wherein the cells express one or more heterologous gene(s).

73. The process of claim 72, wherein the one or more heterologous gene(s) encode a protein which has an immunomodulatory effect.

74. The process of claim 73, wherein the protein is a cell surface receptor.

75. The process of claim 74, wherein the protein is Fas-ligand.

76. The process of claim 72, wherein the one or more heterologous gene(s) express a dominant negative form of an endogenous protein.

77. The process of claim 73, wherein the protein is an antigen target for the immune system, such as an autoantigen, a tumour antigen, or a foreign antigen.

78. The process of claim 64, wherein the cell co-expresses two or more heterologous genes.

79. The process of claim 78, wherein one of the heterologous genes prolongs the life-span of the cell.

80. The process of claim 79, wherein the gene is an anti-apoptotic gene.

81. The process of claim 78 or 79 wherein the gene encodes FLIP or bcl-2.

82. The process of claim 64, in which one or more endogenous gene(s) have been inactivated.

83. The process of claim 82, wherein the inactivated endogenous gene(s) comprise any of: B7-1, IL-12, and the p35 or p40 subunit of IL-12.

84. The process of claim 71, wherein the embryonic stem cells are transfected with at least one gene which is expressed in the dendritic cells.

85. The process of claim 84, wherein the gene is under the control of a promoter which initiates or upregulates gene expression on maturation of dendritic cells.

86. The process of claim 84 or claim 85, wherein the gene is a reporter gene which expresses a detectable product in the dendritic cells.

87. The process of claim 86, wherein the gene encodes a fluorescent product.

88. The process of claim 87, wherein the gene is the GFP gene.

89. The process of claim 71, wherein the ES cells are genetically modified so as to inactivate at least one copy of at least one gene.

90. The process of claim 64, wherein the recovered immature dendritic cells are substantially pure.

91. The process of claim 64, wherein the cells are lymphoid.

92. The process of claim 64, wherein the cells are myeloid.

93. The process of claim 64, wherein the cells are human.

94. The process of claim 64, wherein the ES cells are derived from a mouse strain such as CBA/Ca or C57BI/6.

95. The process of claim 94, wherein the ES cells are from the ESF116 cell line.

96. A substantially pure population of immature dendritic cells obtainable by the process of claim 64.

97. A pharmaceutical composition comprising the population of claim 96 and a pharmaceutical excipient.

98. A method of treating a patient by immunotherapy which comprise administering to a patient an effective immunotherapeutic amount of the population of claim 96.

99. The method of claim 98, wherein the immunotherapy comprises immunostimulation.

100. The method of claim 99, wherein the immunostimulation comprises tumour immunotherapy or vaccination against infectious agents.

101. The method of claim 98, wherein the immunotherapy comprises down-modulation of a detrimental immune response.

102. The method of claim 101, wherein the down-modulation of a detrimental immune response is in the treatment of autoimmune disease or allograft rejection.

103. The method of claim 98, wherein the immunotherapy comprises altering dendritic cell function.

104. The method of claim 98 or claim 103 wherein the immunotherapy comprises inducing a Th1 to Th2 immune deviation.

Remarks

The parent PCT application includes claims 1-63. By amendment herein, claims 1-63 have been canceled and claims 64-104 have been added.

The new claims present the subject matter of claims 1-63 in form for U.S. prosecution; no new matter has been introduced.

Amendments to Specification

The specification has been amended to include Applicants' claim for foreign priority, to include an Abstract on a separate page, and to include section headings where appropriate.

No new matter has been added. A copy of the amended paragraphs of the specification, with markings showing the changes, is annexed hereto.

Respectfully submitted,

Date: May 4, 2001

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VERSION WITH MARKINGS TO SHOW CHANGES MADE
Annex to Preliminary Amendment filed May 4, 2001

bolding with underlining indicates added text

A. In the specification:

1.) Page 1, the following new paragraphs are added following the title, and prior to original paragraph 1.

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Detailed Description of the Invention --

4.) Page 25, paragraph 1 (line 1) is replaced with:

-- **Claims**

We claim: --

5.) A new page 29 (annexed hereto), containing the abstract, is added.

B. In the claims:

1.) Claims 1-63 are canceled, without prejudice.

2.) New claims 64-104 are added.